Friday 24 Aug

Reznikoff's Goal: Make us all professional biotechnologists in 25 days

point mutations
deletions
insertions
inversions

allosteric proteins - regulation

Chemistry discussing alkyls & ethenes of H-bonding in base pairing

Key pt - Base pairing is the means of information transfer

complementary - will they pair?
(don't use homologous to mean complementary

the major groove/minor groove (resides) & the info that contains - there's other info.

Deoxyribose - H @ 2'
Ribose - OH @ 2'

DNA vs. RNA : (Generalizations - not 100% gospel)

deoxyribose vs. ribose
double stranded vs. single stranded (yes oversimplification

Thymine = 5-Me-uracil

TA, GG
CT = pyrim
AG = purines
} vs. AG
CU = uracil

Thymine Adenine
Cytosine Guanine
Uracil

GGGGG AAA CCCCC

A AA
G C
G C
G C
G C
G C

have to lose 3 for the loop
Single stranded can ∴ have internal base pairing & demonstrate this 2° structure

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Source: https://www.industrydocuments.ucsf.edu/docs/qhwk0001

stringent - allows only mut-formed

relaxed - allows some mismatches

"the equivalent of an organic chemist — a machine"

Assays to detect that yes in fact do have SS:
buoyant density - uses binding of Cs salts
frictional coefficient - sedimentation
sensitivity to various nucleases
Δ O.D. predicted from breakdown in stacking

Processive enzyme (processive? lecturer doesn't spell)
does not dissociate readily - stays associated w/ substrate + product
Assumed that RNA polymerase is pro/processive

Will not discuss transcription termination in the class

Mutants which can be isolated ~~+ studied~~ + studied here almost always mutation in non-essential function — otherwise can't get cells to study 'cause they die

Promoters have several aspects in common:
[Nomenclature: in direction of transcription = + = downstream
opp. " " " = − = upstream
∃ no 0 ⇒ −1 beside +1]
−10 is TATAAT and conserved } from start site
−35 = TTGACA

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Different RNA polymerases recognize different promoter sequences
yeast vs E. coli, etc.

RNA polymerase: 2 α subunits, a β, a β', and the σ_{70}
500K = MW
subunits are big
active site on β or β' $\alpha\alpha$, β, β' = necessary
σ subunit part of promoter recognition — $\therefore$ σ is what has to Δ for recog of other promoters

related w/ σ = MW
σ 32 = heat shock
σ 54 = transcribes 2 groups of genes: N₂ metab + fermentation pathways

Recog- Me on Thymine — definition in groove for promoter recognition — promoter AT rich — Me can be probed by side chains in RNA polymerase
(theory! — not clear to me that this can be stated as fact)

IPTG — isopropyl .. galact ... — non-metabolizable molecule used as "inducer"

Think @ this inducer/repressor interaction & how it gets turned off — answer by Reznikoff was that the interactions were not covalent and eventually [ ] ↓ and it ~~was~~ reversible

3 steps of translation
① Initiation:
② Elongation
③ Termination — important here rather trivial; directly related to code

Mis-sense mutation: Δs codon so diff amino acid goes in
non-sense = termination
Each codon has a separate tRNA
All proteins don't have Met on end

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Source: https://www.industrydocuments.ucsf.edu/docs/qhwk0001

common names:
- Amber mutation — UAG — named after translator of discoverer's name
- UAA — ochre
- UGA — umber

mRNA usually has stuff after termination codon

AUG can mean Met or formylMet; depends on whether it's at beginning (formyl) or in middle (Met)
Shine/Dalgarno sequence (= start seq) before AUG
⇒ start here ⇒ formyl Met
For eukaryotes, first avail AUG is for formyl-Met

Code is read in reading frames - have specific stop + start, but no punctuation

Frameshift mutations — same as Δ in reading frame

In order to genetically modify an org, the introduced material has to be heritable. ∴ it must replicate on its own or become associated with cell's machinery.

Initiation of DNA replication is key step
DNA replication is highly regulated. ~~DNA replication is minimized~~
Frequency of DNA replication is minimized
Primer is short RNA - think @!

TAQ Polymerase - Active @ high temperature (70-80°C)

Reverse transcriptases come from retro-virus - AIDS is a retrovirus. Has RNA genome - makes DNA from RNA & then incorporate DNA into genome of host.

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Source: https://www.industrydocuments.ucsf.edu/docs/qhwk0001

Enzyme tool kit: DNA Pol I
TAQ Pol
Klenow
Reverse transcriptase
Terminal transferase
Alkaline phosphatase
Polynucleotide kinase

Nucleases - Bal31, S1
Restriction enzymes
Ligase

Ways to inactivate enzymes: (before next step.)
① Δ
② organic treatment
③ Dep mod - diethylpyrocarbonate
④ chelating agents

2x comments @ lobbying Congress for research funds

The special class of nucleases called restriction enzymes & ligase are most imp.

Defined restriction/modification in terms of LT2, E. coli B and 2B. Restriction enzymes recognize foreign DNA & cut it up — Molec biologists capitalize on this.
Modification function is how cell keeps itself from suicide

"People who do molecular biology are on computer 20% of time"

Polynucleotide phosphatase = blocking group

Properties of vectors: (Exceptions to each)
① ori dent replication structure
② easily manipulated: cut prior to insertion
readily purified
want to be able to get a lot
set into appropriate host cell
③ biological construct - be able to find "transformed" cell - a selection procedure Ex: antibiotic resistance
④ origin of replication & the selection procedure must be compatible w/ host

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Source: https://www.industrydocuments.ucsf.edu/docs/qhwk0001

Phage ≡ ed = ~~redacted~~ virus

λ is used just to get collection of DNA which can be screened by hybridization to select gene which will then be excised, etc

library

M13 is valuable because it yields ss DNA which is compatible w/ Sanger sequencing

- ∃ certain biological limitations. Plasmid w/ "lot" ∃ foreign DNA hard to handle - it's big. Also need high enough ⊕ copy #. Something @ cells w/o overriding cells ctl.

Sources of foreign DNA

① genomic DNA = chromosomal DNA

② cDNA = copy from the message
will not include introns or upstream of start and downstream from start
representation should ~~start~~ demonstrate relative abundance of that message

Library is collection of clones of DNA molecules from complex mixture

③ synthetic DNA

Oligo dT columns: Based on fact messages have polyA tails. Message will ∴ bind to column w/ run of Ts. Regnitoff likes magnetic bead technology

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Source: https://www.industrydocuments.ucsf.edu/docs/qhwk0001

- Cloning done:
  Don't get plaques from λ unless you've incorporated foreign DNA

- Almost all eukaryotic mRNA has poly A tail at 3' end. Prokaryotic mRNA does not have it.
  Poly A tail is post translational modification

PCR: Target sequences, make primers, denature, anneal primers, → elongate

amplification region ≡ed by end 3' primers

there's also an alternate being incorporated cause of the 5' end in the primer

limited to 3000 - 4000 bp - PCR can't make chromosomes

- Use of nucleic acid probe technology to detect if sequence related to pathogen is found there
  Use PCR to diagnose AIDS infection in newborn - all will have Ab ~~of~~ but may not from infected mom
  have the virus

- Redox potentials in bacteria + absence of disulfide bonds in protein expressed there.
  Refolding experiments

- Buell: get the most imp technique in his lab

Buell example - some mutations are affecting rate of synthesis, others rate of degradation

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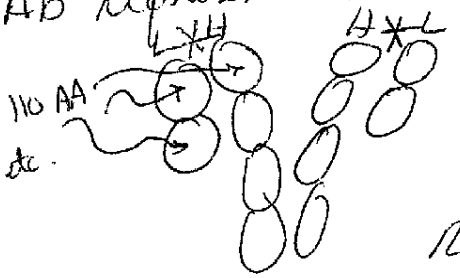
Source: https://www.industrydocuments.ucsf.edu/docs/qhwk0001

Ab representation:



L = light
H = heavy
should be symmetrical subunits!
* = antigen binding site
Recombinant antibodies may only be 2 chains (1 L, 1 H)

∃ "V" cells and "C" cells
C = constant
V = variable?

∃ many V region genes but only one C
Do by PCR (Wanter PNAS paper)
only 3 regions are where the variability is in (the 110 aa). ∴ can take PCR approach called hypervariable — and at least one corresponds to antigen binding site + that's why they must vary...

transfect = to eukaryotic cells what "transform" means for prokaryote. "Transform" in eukaryotes means tumorigenesis.

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